Attorney Docket No.: 205-009US1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	David E. Wolf	Art Unit: 1743
Serial No.:	10/698,591	Examiner: Ramillano
Filed:	October 31, 2003	Confirmation No.: 1643
Title:	SEMIPERMEABLE SENSORS FOR DETECTING ANALYTE	

MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF DAVID E. WOLF, PH.D.

I, David E. Wolf, Ph.D., state and declare as follows:

1. I am the inventor named on the above-captioned application and the Chief Technology Officer at Sensor Technologies LLC.

2. Prior to March 11, 2002, I had, Kate Sears, a then research associate of Sensor Technologies LLC, prepare a series of sensor beads. The sensor beads included an alginate core that included a fluorescence energy donor and a fluorescence energy acceptor. The alginate core was surrounded by a semipermeable poly-L-lysine membrane. The semipermeable coating was surrounded by a biocompatible coating formed from alginate. Ms. Sears' work is reflected in laboratory notebook S-063, pages 36, 37 and 46, copies of which are attached hereto as Exhibit A.

3. The entry "Cy3.5-TH-071" refers to fluorescence donor dye molecules from Amersham BioSciences, Cardiff Wales. The entry "Cy5.5-C-059s" refers to fluorescence acceptor dye molecules from Amersham BioSciences, Cardiff Wales.

4. The lot of sensor beads reflected on laboratory notebook pages 36, 37 and 46 have been identified internally at Sensor Technologies LLC as Sensor Bead Lot CC.

5. The sensor beads of lot CC were coated with semipermeable poly-L-lysine having a weight average molecular weight of 9800 Dalton (lot number 127H5912). I have reviewed records at Sensor Technologies LLC and determined that the lot of poly-L-lysine used to prepare the sensor beads of lot CC was obtained from Sigma-Aldrich,

Inc., and each lot of poly-L-lysine had a polydispersity index greater than 1. In particular, lot number 127H5912 had a polydispersity index of 1.18.

6. The sensor beads of lot CC had an approximate diameters of 2 millimeters.

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under section 1001 Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent on which this statement is directed.

Further I declare not.

Date: September 20, 2007

David E. Wolf, Ph.D.
Chief Technology Officer

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EXHIBIT A

REDACTED

Prepare PLL Stock Solution

→ Allowed PLL to warm to room temp (L# 274912)
→ weighed out 120.08 mg PLL into 50 mL conica tube
→ Added 12 mL HEPES | SALINE | CA into tube
→ Placed on rocker for 10 min

Prepare PLL coatings

→ To each of two conical tubes added the following

	PLL stock	HEPES | SALINE | CA pH 7.4
Tube #1	6.0 mL	22.0 mL
Tube #2	4.0 mL	14.0 mL

→ sterile filtered both coating into 50 mL conical tubes
→ placed in water bath @ 37°C

Prepare Chemistries

→ To a 5 mL vial placed 462 µL Cy5.5-C-0695
→ Added 77 µL Cy3.5-TH-071
→ Added 462 µL HEPES | SALINE buffer pH 7.4 (From 6/30 batch)
→ Added 1.0 mL 2.85% alginate soln (From 7/6 batch)
→ covered w/ foil and placed on rocker for 5 min

Make Beads

→ Drew above chemistries into a sterile 3cc syringe w/ a 14g catheter
→ Removed all air bubbles from the sample
→ Replaced 14g catheter w/ 22g catheter
→ Placed syringe on syringe pump @ 5mL/hr setting
→ Allowed syringe to drip into sterile 50 mL Erlenmeyer containing 30 mL HEPES/1.5% $CaCl_2$ soln

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Read and Understood By

K Sears — Signed — REDACTED — Date
Diane Ellis-Busby — Signed — REDACTED — Date

REDACTED

- Allowed beads to soak in solution for 20 min
- transferred to 50 mL conical tube, allowed beads to settle to bottom
- pipette out liquid from beads and discarded
- rinsed 4x w/ Hepes/Saline/Ca solution pH 7.4
- Removed final rinse and added 1st PLL coating (Tube #1)
- Placed in water bath at 37° w/ agitation for 15 min
- Removed liquid and washed 4x w/ HBS/Ca buffer
- Kept 4th wash on beads and allowed to sit at room temp for 40 min
- Removed buffer and added 2nd PLL coating (Tube #2)
- Placed in same water bath w/ agitation for 15 min
- Removed soln with pipette, and washed 4x w HBS/Ca soln
- transferred beads in buffer to 5mL tube, covered w/ foil and stored @ 4°C

* All amounts of substances used were calculated by the Bead Mixture Calculator (shown below) *

Bead Mixture Calculator

Instructions: Values shaded in blue are to be replaced as needed. Values shaded in green are calculated by the worksheet.

	Lot Number	Protein Conc. (mg/ml)	Dye/Protein Ratio	Bead Lot
Donor	Cy3.5-TH-071	2.70	0.66	CC
Acceptor	Cy5.5-C-0596	1.24	0.21	

FRET Proportions (%)	
Donor	5
Buffer	95
Acceptor	30
Total Vol	130
Desired Volume of Beads (mL)	2.00

Bead Recipe	
Volume Donor (µL)	77
Volume Acceptor (µL)	462
Volume Buffer (mL)	0.462
Volume Alginate Stock (mL)	1.000
Alginate Lot	411-256-06 2.05% 7/600

PLL Coatings	
First: PLL stock (mL)	6.0
First: Buffer (mL)	22.0
First: Total Vol (mL)	30.0
Second: PLL stock (mL)	4.0
Second: Buffer (mL)	14.0
Second: Total Vol (mL)	20.0
Total PLL Stock Required (mL)	10.0

Conc. of Donor Protein (ug/mL)	103.8
Conc. of Donor Protein (Molarity)	1.66E-06
Conc. of Acc. Protein (ug/mL)	286.2
Conc. of Acc. Protein (Molarity)	2.75E-06
Conc. of Donor Dye	1.03E-06
Conc. of Acceptor Dye	5.70E-07
PLL Lot	127H5912

Information		Notes
Bead Lot:	CC	22 gauge catheter used to make 2 mm beads
Date:	REDACTED	
Hardening Time (min)	20	
1st PLL (min) / (temp)	15/37	
2nd PLL (min) / (temp)	15/37	
Time between (min)	60	

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Read and Understood By

K. Sears — Signed
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Diane Ellis-Busby — Signed
REDACTED — Date

PROJECT 2nd coating on 42 Lot CC beads
Fret Assay Cu55-C-ados /gels, 3.5 ~~0711~~ KS

Notebook No. S-063
Continued From Page X

REDACTED

→ changed buffer on glucose exposed Lot BB and Lot CC beads from 7/24

2nd alginate coating layer (Lot CC)

→ placed 42 beads from Lot CC in a sterile petri dish in hood
→ seperated beads
→ add one small drop alginate (1mL) / HS (1mL) pH 7.4 mix ~~[illegible]~~ KS
→ rolled bead in alginate mix
→ picked up bead with tweezers and placed on clean spot on dish
→ picked up bead again and tapped on edge of tube containing 1.5% $CaCl_2$ in Hepes soln until dropped in.
→ Repeated procedure for each bead
→ allowed to harden for 20 min
→ seperated beads into aliquots of 2 beads per .5mL buffer

REDACTED

Continued on Page 47

Read and Understood By

K. Sears — Signed | REDACTED — Date | Diane Ellis-Besley — Signed | REDACTED — Date